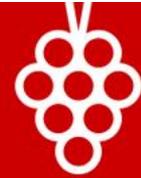


Microbial Terroir: Isolation of Natural Yeasts from Vineyard/Winery Operations for Use in Wine Production

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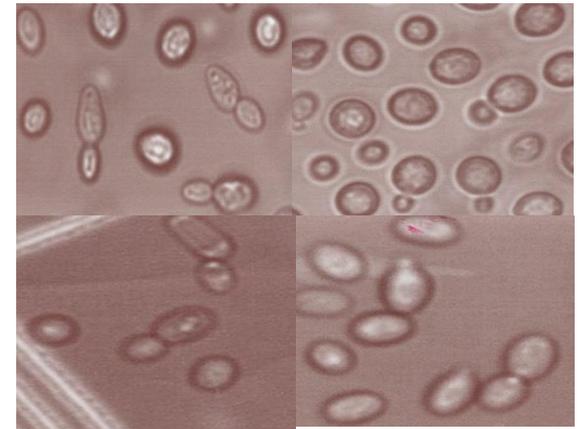
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Microbial Terroir



- Growing interest worldwide, including Canada, to further differentiate wines by expressing a region's microbial terroir
 - Use the natural yeast on the fruit to ferment wine
 - Add complexity to wine due to contributions of many yeast species
 - Hall et al, 2011; Jolly et al, 2014; Scholl et al, 2016; Morgan et al, 2019; Kelly et al, 2020; McCarthy et al, 2021; Bunbury-Blanchette et al, 2022
- **Risks: inconsistency, off flavours, oxidation faults**



Commercial *S. cerevisiae* offer control over a fermentation, but is regional identity sacrificed?



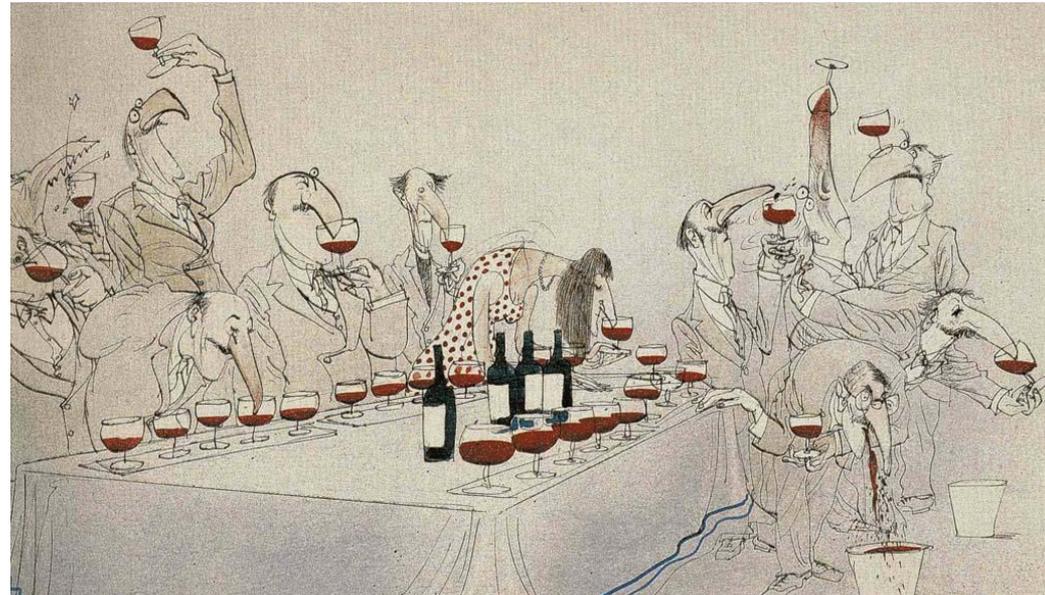
- Most commercial yeast on the market are of the species *Saccharomyces cerevisiae* although a handful of non-*Saccharomyces* yeast are entering the market
 - *Saccharomyces cerevisiae*:
 - alcohol tolerant
 - can complete fermentation
 - selected for oenological performance and flavour profile of resultant wines
- Most *S. cerevisiae* were selected from European countries, may not be the best performing yeast for our cool climate regional wines

Commercial Yeast versus Spontaneous Fermentation



Commercial yeast

-Wines may not exhibit characteristics of the local microflora but there is more control over the ferments, less risk of stuck fermentations and off flavours due to yeast strain



Spontaneous Fermentation

-Some winemakers like the complexity of working with indigenous, natural yeast but not all indigenous yeast can complete a fermentation, and many can produce off flavours
-More difficult to control a natural, spontaneous fermentation because you are not sure what yeast will be present

Can winemakers differentiate their wines with their own yeast but still attain consistency and quality?



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- YES, we have the tools to isolate your own yeast
 1. Select yeast from a spontaneous fermentation, focus on isolates that are able to complete a fermentation
 2. Identify the yeast isolates to species, then strain level
 3. Trial isolates from culture preparations in small scale ferments to select best performing yeast
 4. Utilize a local yeast company to grow up the cultures in paste form for commercial fermentation

Step 1. Spontaneous fermentation

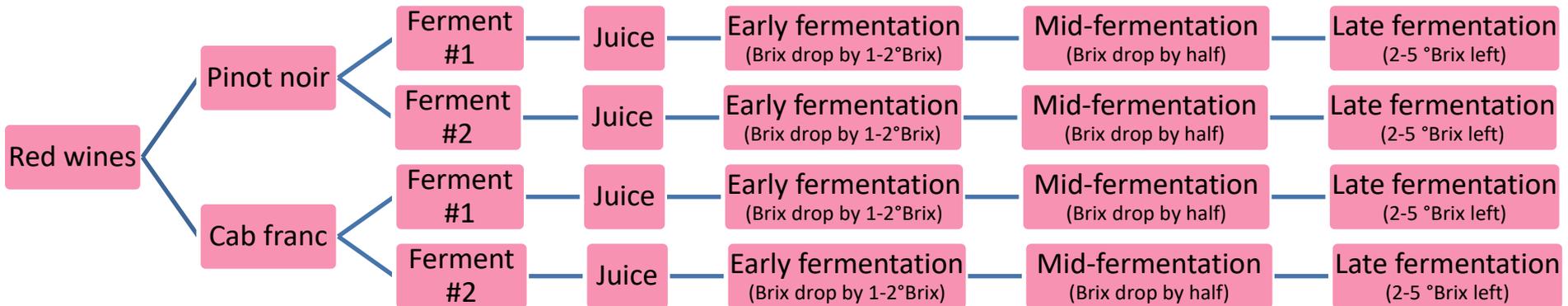
Trial with local winery,

Experimental design: Red wine



Sampling points

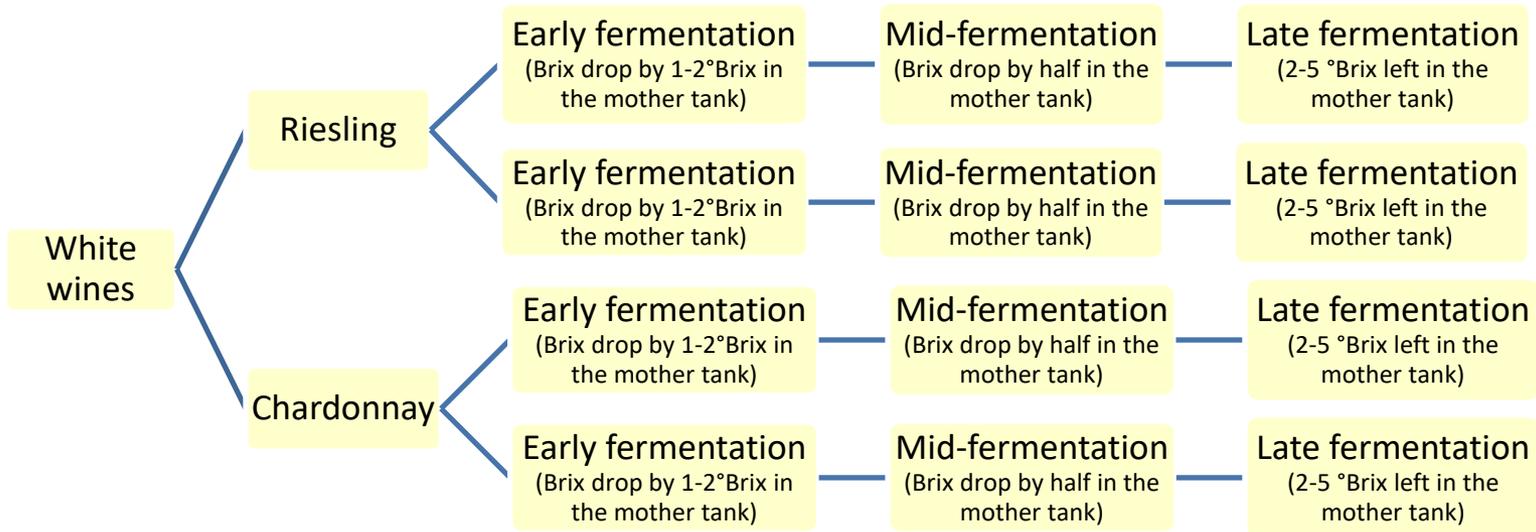
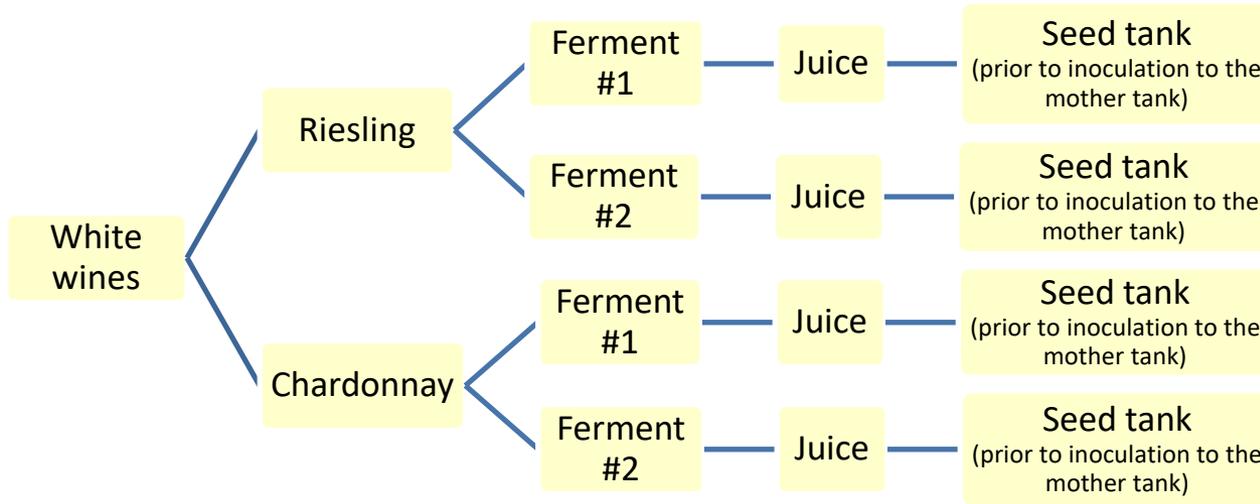
(at each sampling point, 3 X 50 mL samples are taken)



- Ferments were performed in duplicate at the winery
- Sampled at 3 different stages of the ferment:
 - early, middle and end (triplicate sampling at each timepoint, 3 X 50 mL)
- Only identified and selected yeast from end stages of fermentation

Trial with local winery

Experimental Design: white wine



Step 2. Yeast isolation and identification

Chardonnay, late stage sampling

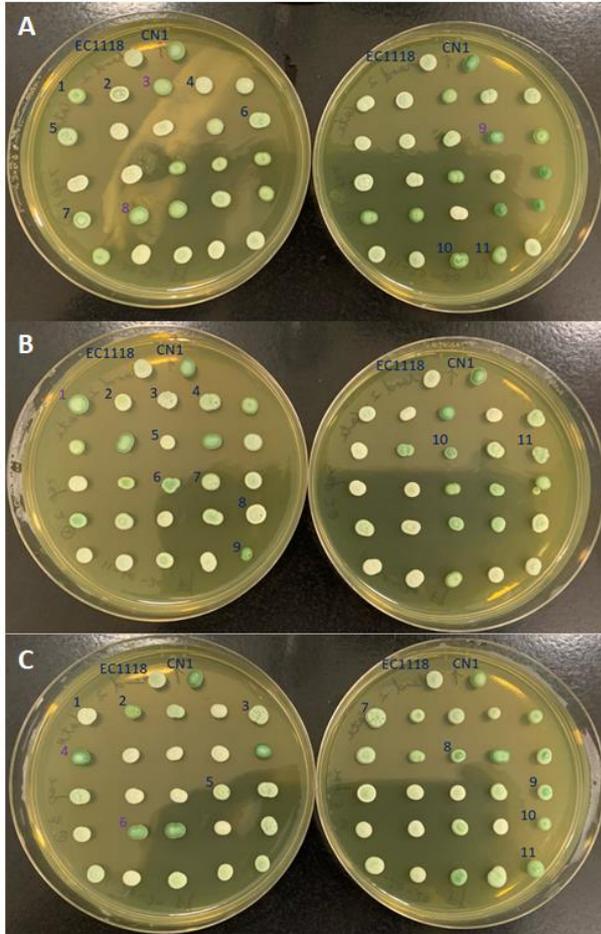


Figure 1. Results of preliminary identification of colonies isolated from the 2nd Chardonnay fermentation

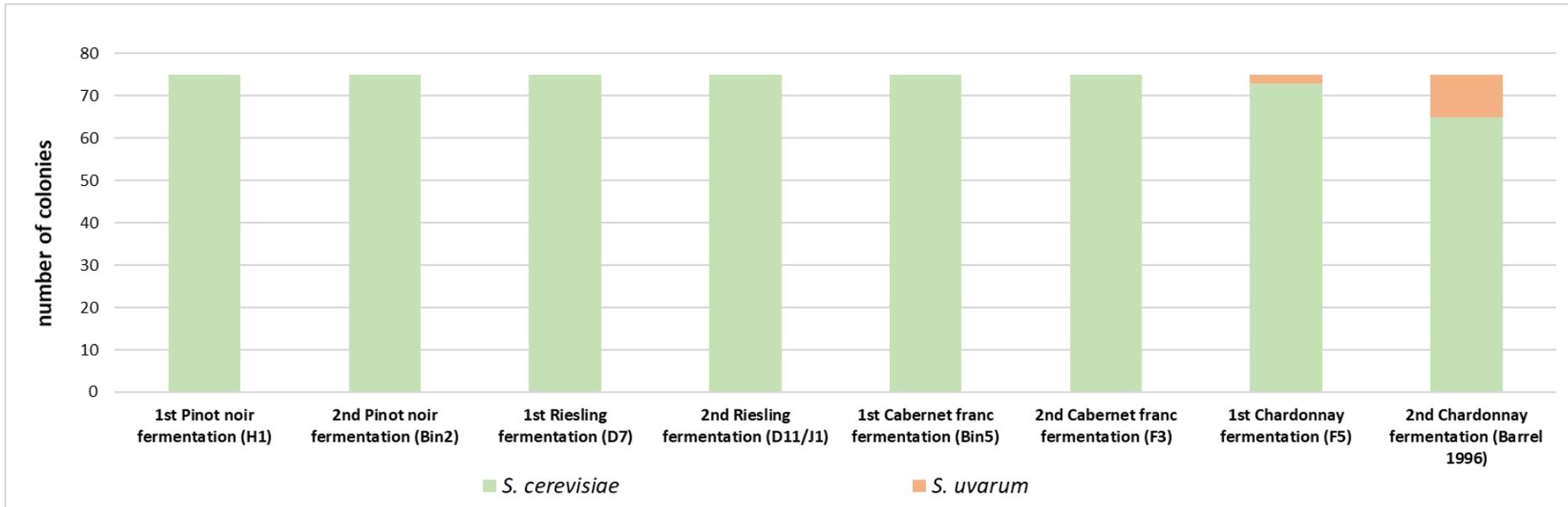
-Green colonies *S. uvarum*

-Cream colonies *S. cerevisiae*

-25 isolates were selected from each YPD plate, replated on WLN differential media for preliminary identification as *S. cerevisiae* or *uvarum*

-Yeast identification confirmed using molecular techniques (PCR)

Comparison of yeast isolates across all fermentations



- 600 isolates characterized
- Of the 150 isolates from Chardonnay, 12 were *S. uvarum* unique to the winery 
- 588 were *S. cerevisiae* 

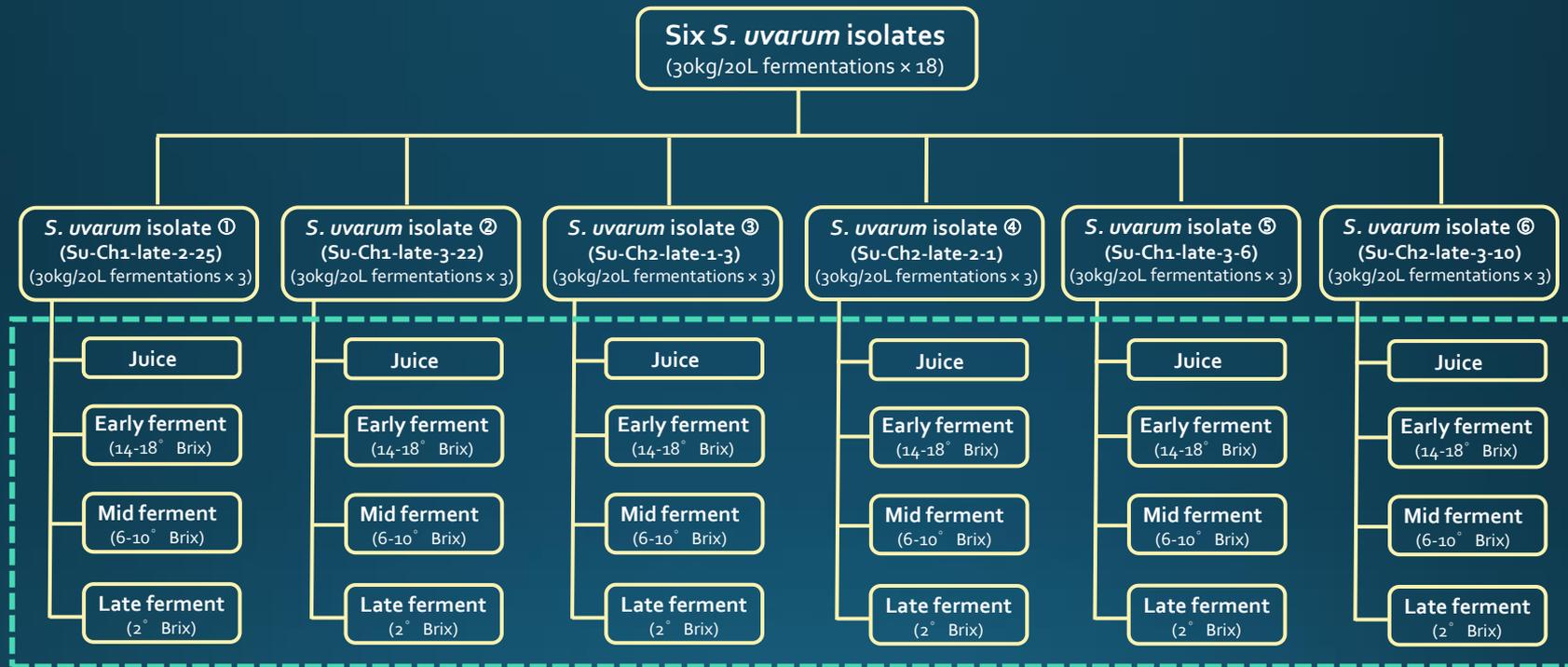
Step 3: Test small scale ferments

-6 isolates of *S. uvarum* retested



- All *S. uvarum* isolates were unique to the winery, the winery had never used the one commercial *S. uvarum* strain on the market (Velluto[®] BMV58 (Lallemmand, Montreal, QC, Canada)).
- Tested 6 of 12 isolates for implantation efficiency into fermentations (Cabernet franc juice)
- Yeast paste cultures were prepared from each yeast isolate by Escarpment Laboratories (Guelph) to use in fermentations
 - No active dried culture process available for new yeast isolates
 - Escarpment Labs routinely prepares cultures for the brewing industry
 - Service accessible to wineries for culture preparation

Experimental Schematic Diagram for *S. uvarum* Fermentation Trial



- There are six fermentation treatments in total and each treatment will be performed in Cab franc juice in triplicate.
- All fermentations are daily monitored for sugar consumption and ethanol via Foss.
- Samples will be taken for yeast analysis once the sugar falls into the above-specified ranges.

Microbial analysis of time-course samples from Cabernet Franc fermentations



- 6 yeast isolates were used for inoculation into cabernet franc
- inoculated with *S. uvarum* paste cultures prepared by Escarpment Labs
- Samples from triplicate fermentations were plated on YPD with antibiotics to kill any bacteria then 25 colonies from each YPD plate were randomly selected and sub-plated on WLN plates
- Colonies were classified as *S. cerevisiae*, *S. uvarum* or other species on WLN plates, counted and the average colony numbers from the triplicate fermentation samples are shown in Figure 2.

Implantation of *S. uvarum* isolates into Cabernet franc fermentations

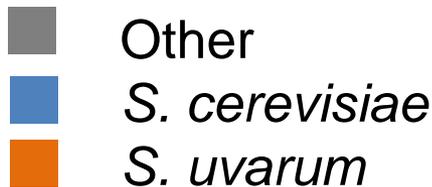


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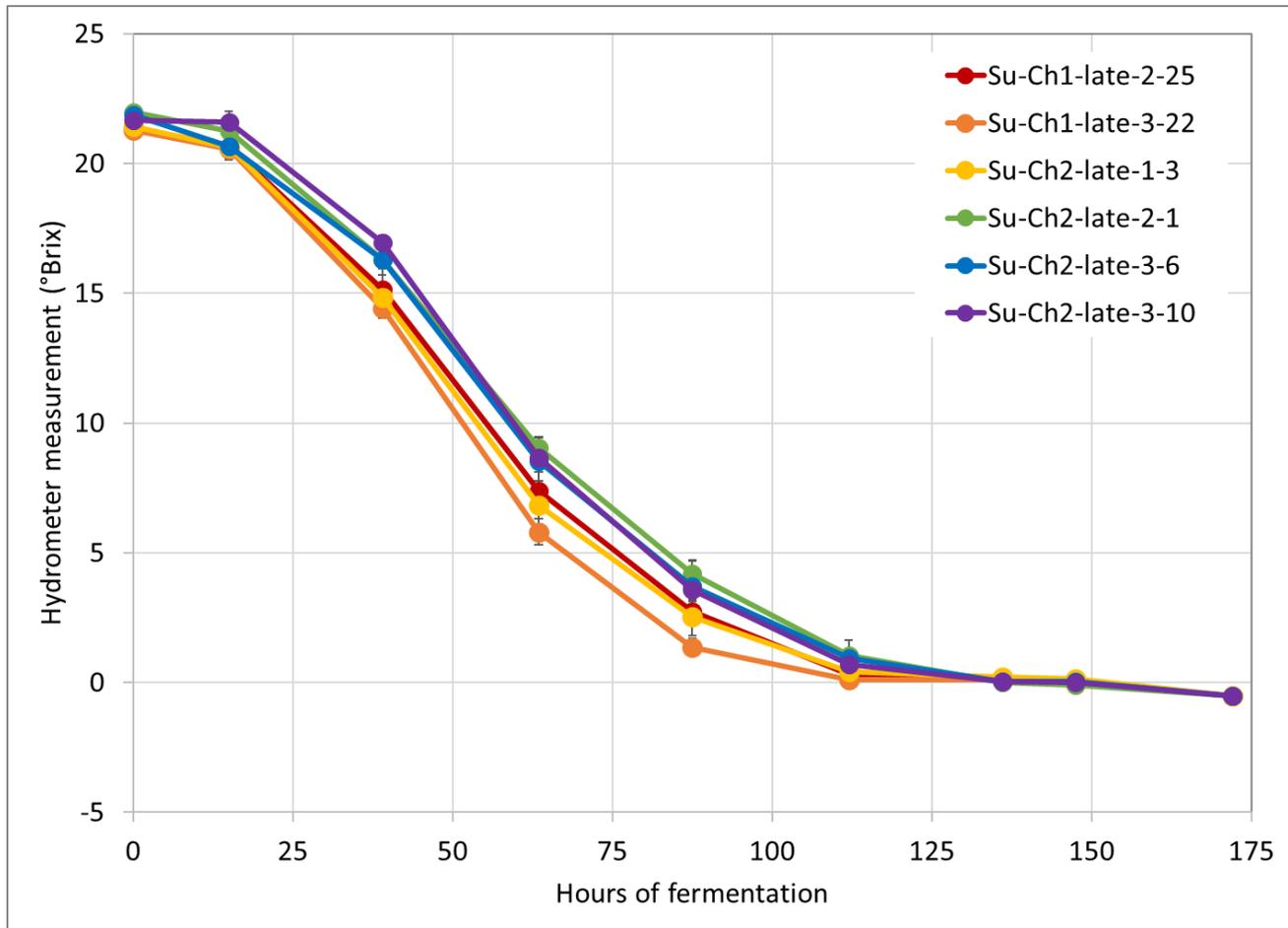
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Figure 2. Microbial analysis of time-course samples from Cabernet franc fermentations inoculated with *S. uvarum* paste cultures.



Fermentation performance



-All isolates fermented to dryness
-all allowed spontaneous MLF to proceed
-no faulted wines
-formal sensory evaluation of wines not possible through COVID but the winemaker happy with informal tasting and will proceed with commercial trials

Strain identification by microsatellite analysis showed 3 different strains of *S. uvarum* from the 6 isolates



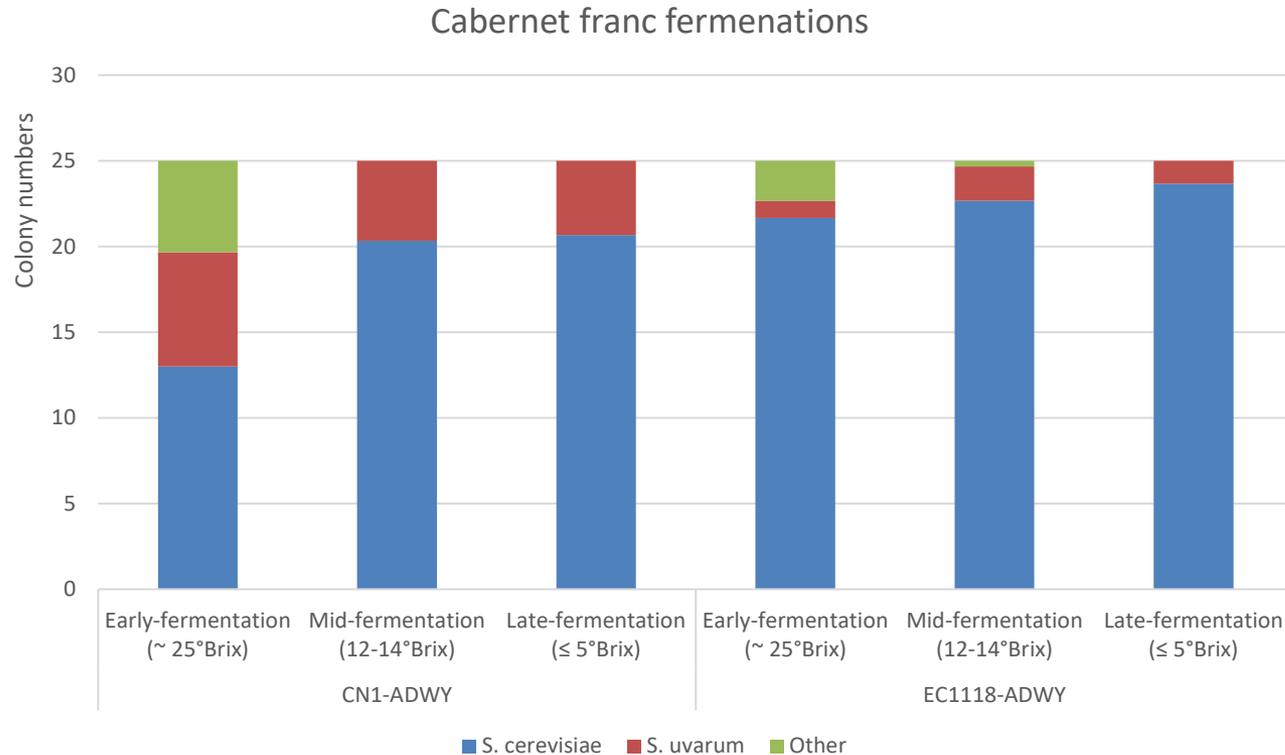
Yeast isolate code	Carboy code	Yeast strain type
Su-Ch1-late-2-25	1A	Strain A
	1B	
	1C	
Su-Ch1-late-3-22	2A	Strain B
	2B	
	2C	
Su-Ch2-late-1-3	3A	Strain C
	3B	
	3C	
Su-Ch2-late-2-1	4A	
	4B	
	4C	
Su-Ch2-late-3-6	5A	
	5B	
	5C	
Su-Ch2-late-3-10	6A	
	6B	
	6C	

Step 4: Commercial fermentation



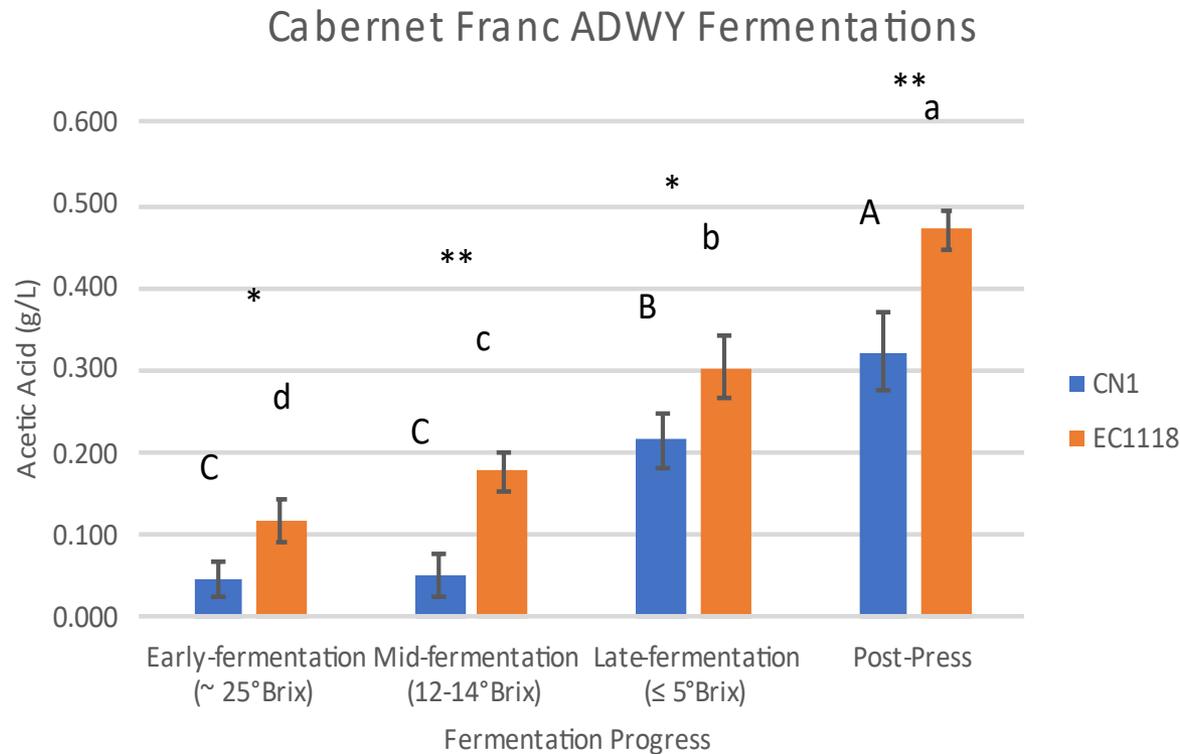
- Plan to trial *S. uvarum* isolates from this project at the commercial winery this fall
- We have successfully trialed another *S. uvarum* isolate we have been characterizing for a few years in fall 2021 - CN1
- That yeast was prepared by Lallemmand as an active dry culture and performance compared to EC1118
 - Previous work had shown CN1 *S. uvarum* Shifted the sensory profile of Cabernet franc appassimento wine towards increased black fruit flavour and aroma
 - Reduced sourness and astringency vs. *S. cerevisiae* EC1118 commercial yeast

S. uvarum CN1 implanted into the ferment and competed with *S. cerevisiae*



Juice had a substantial background of *S. cerevisiae* due to pre-fermentation processing of grapes but *S. uvarum* still established itself

Commercial fermentation of ADY preparation of locally isolated *S. uvarum* CN1 fermenting Kiln dry fruit



- CN1 was successfully prepared in an active dry form, implanted and completed commercial fermentations of appassimento wine with significantly lower acetic acid production

Summary



- **Services are now available through CCOVI for winemakers to efficiently isolate their own natural yeast, store the cultures and have them prepared in a paste format through Escarpment Labs for use in commercial ferments as opposed to an active dry format**
- **Yeast isolated from a vineyard/winery operation may allow the winery to differentiate their wines in the marketplace by taking advantage of yeast biodiversity**
- **Natural yeast isolates are one more tool available to the winemaker, and sometimes these yeast have unique positive properties not present in commercial selections (e.g. CN1)**

Acknowledgements



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- Lallemand
- Escarpment Laboratories

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Thank you!



Questions?

Cheers!

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